IMMUNE RESPONSE IN NEUROCYSTICERCOSIS: SOLITARY VERSUS MULTIPLE NEUROCYSTICERCOSIS

Dissertation submitted to the Dr. M.G.R Medical University, Chennai, Tamilnadu in fulfillment of the DM – Neurology examination in August 2009.

CERTIFICATE

This is to certify that this study **"Immune Response in Neurocysticercosis:Solitary versus Multiple Neurocysticercosis"** is a bona fide work of Dr. Sampathkumar. C and is submitted in fulfillment of the DM – Neurology examination conducted by the Dr. M.G.R Medical University, Chennai, Tamilnadu in August 2009.

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INTRODUCTION

Neurocysticercosis (NCC) is caused by the larval stage of the pork tapeworm, *Taenia solium* and is a major public health problem, especially in developing countries where it is endemic. It is the most common parasitic infection of the central nervous system (CNS) and cause of symptomatic seizures (late onset seizures) in the disease endemic countries, causing major morbidity and mortality.

T solium infections are those of antiquity. Tapeworms and their treatment were discussed in ancient Egypt and possibly referred to in ancient Indian literature. The earliest description of cysticercosis are that of vesicles in the corpus callosum by Paranolus in 1550. *Taenia solium* infection causes two diseases in humans, "*Taenia*sis", the intestinal infection with the tapeworm and "Cysticercosis", the larval infection of the tissues. The serious *T solium* infections are those of the central nervous system and of the eye.

Poor sanitation and animal husbandry practices are found to be risk factors for NCC. Risk factors for neurocysticercosis of poor sanitation, free roaming pigs and non-regulated sale of pork, exist in all countries where *T solium* infections prevail. Yet in South and Latin America, Sub-Saharan Africa

and most countries of South East Asia, NCC is predominantly a multi cyst infection while in India NCC is mainly a solitary cyst infection. It is not clear why the magnitudes of infection are so different.

T solium eggs ingested by man, release hexacanth embryos in intestine, which through circulation reaches target organs resulting in cysticercosis. *T solium* ova density in the environment is one of the factors suggested in determining cyst numbers in NCC. Low ova densities are associated with less ova ingestion and the development of solitary cyst NCC. In environments highly contaminated with *T solium* ova the probability of ingesting large numbers of eggs is high and would result in multi-cyst disease. In India, even though the frequency of pork consumption is less than most endemic countries, the prevalence of *Taenia*sis is comparable to other endemic countries; yet solitary cyst disease is more common than multiple cyst disease in India.

It is possible that cyst load in NCC is influenced by host immunity. The number of exposures, genetic factors, host-parasite immune response and the presence of co-infections could modify the immune response and play a role in determining the outcome. In *Taenia* infections the host determines the severity of disease, resistance to and pathogenesis of NCC. The innate immune response induced in early interactions between parasite antigens and immune cells of the host is important in determining whether parasite oncospheres will be cleared or permitted to establish cyst infection. The subsequent host cellular immune response would be important in determining the course of the infection as well as in clearance of the cyst.

It has been shown in animals that immunity against helminth infections requires the induction of a Th1 / pro-inflammatory response while infection is established by induction of a Th2 / anti-inflammatory cytokine response in the host. The cellular immune response has not been clearly defined in human *Taenia* infections especially neurocysticercosis but may also impact on disease profile.

To explore the influence of host cellular immunity and cyst number in Indian patients with NCC, the response of peripheral blood mononuclear cells (PBMCs) to infection specific *T solium* glycoprotein antigens was compared between healthy young adults and patients with multi-cyst and solitary cyst infections. The immune response to N-glycans of the infecting *T solium* glycoproteins was also studied in view of their critical importance for cysticercus antibody recognition. Interleukin-12(IL-12) was measured as a marker of the Th1 response and interleukin-4 (IL-4) of Th2 response. The possible role of nitric oxide in protecting the host against infection and thus contributing to magnitude of cyst load was also examined.

AIMS AND OBJECTIVES OF THE STUDY

AIM:

To determine the role of host cellular immunity in the prevalence of *Taenia solium* solitary cyst infections in India.

OBJECTIVES:

1. To study the Th1 and Th2 cytokine response of peripheral blood mononuclear cells, from patients with solitary NCC, multiple NCC and from healthy controls, stimulated with *T solium* infection specific glycoprotein antigens.

2. To study the nitric oxide response of peripheral blood mononuclear cells, stimulated with *T solium* infection specific glycoproteins to understand the role of macrophages in protecting the host against *T solium* cysticercosis.

REVIEW OF LITERATURE

Biological cycle and characteristics of *Taenia solium*

The pork tapeworm *Taenia solium* belong to the family *Taenidae*. The family *Taenidae* has 11 genera of small to large sized tapeworms. The genus *Taenia* has about 20 species that in addition to *T solium* (pork tapeworm) include *T saginata* (beef tapeworm), *T crassiceps* (rodent tapeworm), *T hydatigena*, *T ovis* and *T pisiformis* (canine tapeworm). Among *Taenidae*, the species that pose health problems to humans are *T solium*, *T saginata*, *Echinococcus granulosus* and *E multilocularis*.[1]

The life cycle of the cestode *T solium* involves two stages: the adult tapeworm, living in the human small intestine, and the larvae or cysticerci, usually located in the muscle of pigs. Humans can be the definitive (intestinal worm, sexual cycle) host or intermediate (neurocysticercosis, asexual cycle) host or both for *T solium*. There is generally one single adult worm, however, there may be several, particularly in cases of habitual ingestion of raw pork meat as seen in some endemic communities.

The adult worm (Fig.1) presents a flat, tape-like shape, reaching as much as eight meters in length. *T solium* is a hermaphrodite. It has no celomic cavity or digestive apparatus, and nutrition occurs through the surface. The excretory system is made of two channels located sideways and lengthwise on the

proglottids, connecting to those in the next proglottid and open to the outside in the last proglottid. The genital pores are located on the side edge of each proglottid, at medium height.

Three portions can be observed: head, neck and body or strobile.

Head or scolex: It is globular in shape, 1 mm in diameter. It has a rostellum, with a double crown of hooks, arranged alternately. It also has four ventoses, being hooks and ventoses its fixation organs to adhere to the intestinal mucosa.(Fig.2)

Neck: Short and thin, measuring 5 to 10 mm long, it is the portion with the highest biosynthetic activity, because the formation of the immature proglottids starts off at the neck.

Body or strobile: It is the longest portion of the parasite and is made of hundreds of proglottids or rings of the following kinds:

Immature Proglottids: Their transversal diameter is longer than the longitudinal one. An incipient genital apparatus can be observed.

Mature Proglottids: Square in shape, follow the immature ones. The female genital apparatus can be seen with a trilobulated ovary, and the male genital apparatus with over 100 testicular masses.

Gravid Proglottids: They are rectangular in shape, with a slightly predominant longitudinal diameter. Most of the male and female genitalia are atrophic, and only the uterus can be seen with 30,000 to 50,000 eggs. The uterus is branched

with an axis alongside the proglottid. It has up to 12 primary branches coming out of it, and smaller dendritic ramifications arising from the primary branches.[2]

Tapeworm

Adult Worm

Fig.1: Adult Worm

Scolex

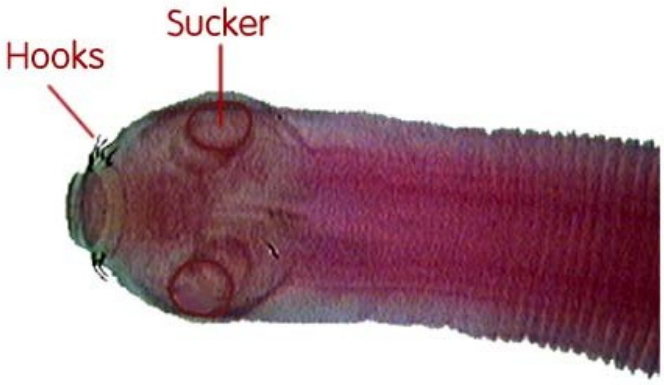


Fig.2: Scolex

Source: CDC – Division of Parasitic Diseases.

Physiology:

T solium has no digestive tract. Food is obtained from the intestine through the body surface, which has microvilli that increase the parasite's surface and enhance the metabolic exchange between the tapeworm and the intestinal environment. Excretion products are eliminated through excretory tubes whose openings are located in the last gravid proglottid of the strobile.[2]

The excretory/secretory products eliminated by the tapeworm to the outside, along with the feces, are useful because they contain antigenic fractions (coproantigens) used for immunodiagnosis through the preparation of specific antibodies and their use in highly sensitive techniques, such as ELISA (Enzyme-Linked ImmunoSorbent Assay).[3]

Life cycle: (Fig.3)

Man is the sole definite host for the adult *taenia*, and the pig is the usual intermediary host for the larval stage. Man can be an accidental intermediary host.

Ingestion of raw or undercooked pork infested with cysticerci leads to *Taenia*sis. Ingested cysticerci are activated in gastric juice, attach to the mucosa of the small intestine, and develop into adult tapeworms. The worm elongates by strobilization, which is a formation of a string of proglottids. The proglottids mature and become gravid. The terminal, gravid proglottids break off from the adult tapeworm and are excreted in feces.

The eggs, eliminated free or inside gravid proglottids in the feces of the individual infected with *T solium* adult tapeworm, contain a hexacanth embryo presenting with six hooks. The eggs are morphologically undistinguishable from those of *T saginata,* making the differentiation difficult. The development of fluoresceine-conjugated specific antibodies against *T solium* eggs helps in species identification. When ingested by the pig, eggs are submitted to the action of the digestive solutions, and release the hexacanth embryos that adhere to the mucosae and then penetrate the intestinal walls reaching blood vessels and are transported to various organs and tissues, including muscle, eye and brain.

Humans complete the life cycle of *T solium* by ingesting infected pork. From an evolutionary standpoint, the location of cysticerci in pig muscle is important, since it permits the completion of the life cycle. Humans like pigs, can become intermediate hosts if fresh vegetables, uncooked food, or water contaminated with human feces is ingested.

The cysticercus is the larval stage of *T solium*, called Cysticercus cellulose by Rudolphi in 1803 given its predilection for conjunctive tissue. The morphology of cysticercus is generally that of a vesicle, but at times, in the brain, this morphology can vary to irregular forms (Racemose form). The infection by cysticerci is called Cysticercosis, human or porcine.[2]

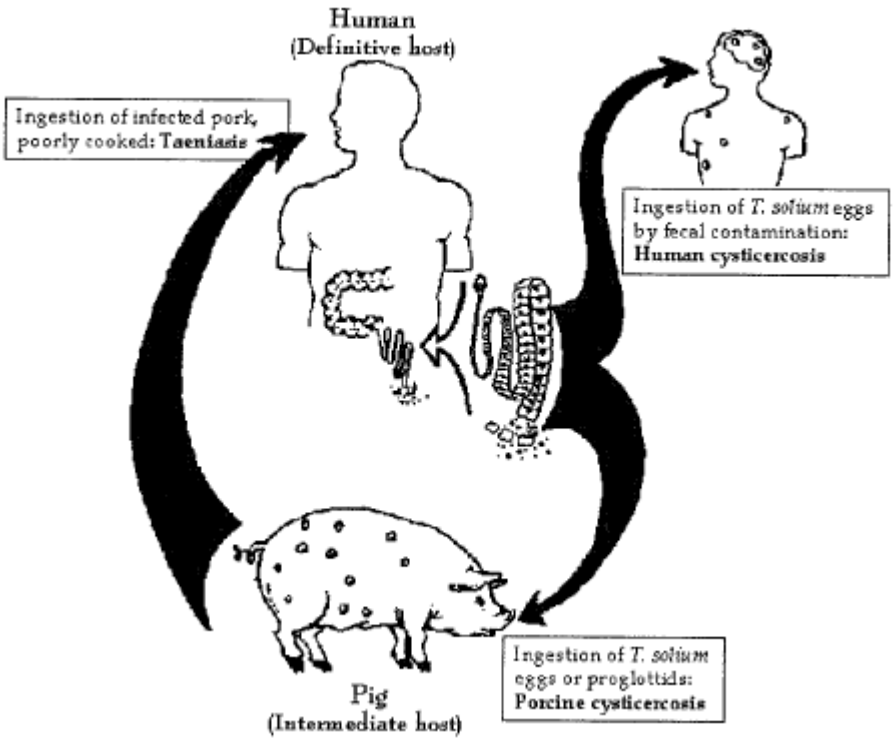


Fig.3: Life cycle of T solium

Source: Clinical Microbiology Reviews, Oct 2002.[4]

Human cysticercosis

Man can ingest *T solium* eggs in contaminated food. Contact with *taenia* infected individuals is believed to be the most probable mechanism for infection along with poor hygienic habits allowing for the contamination of hands by eggs, and thus the likelihood of contaminating the food for the family group. Also, eggs that contaminate short-stem vegetables and/or water for consumption can also be ingested, particularly in areas with poor environmental sanitation and open air defecation.

It is also claimed that intestinal retroperistaltic movements induced in *taenia* carriers by any cause, such as vomiting, would allow for eggs to reach stomach, and here, the action of gastric fluids on the proglottids or egg surface may permit the release of the hexacanth embryos, their penetration into the intestinal mucosa and the completion of the known course, thus causing an "*internal autoinfection*". This possibility would substantiate the presence of 6% of *Taenia*sis in individuals who are currently infected with cysticercosis.

The location of cysticerci in human tissue varies from the subcutaneous location, which is relatively benign, to ocular and central nervous system locations, with a malignant evolution.[2]

Neurocysticercosis

T solium infections of the central nervous system and of the eye are the most serious forms of the cysticercosis in humans that are associated with high morbidity and mortality. Clinical manifestations of neurocysticercosis are highly variable depending upon the number, location and the evolutionary stage of the cysts.[3] The most common manifestation of neurocysticercosis is seizures, focal or generalized, which manifest when cysts degenerate in the brain causing intense inflammatory response by the host.

T solium infections are the most common parasitic infestations of the central nervous system and cause of recent onset seizures in countries endemic for the infection. Epilepsy affects 5 million people in India and is the second most

common chronic neurological condition in the country. Here one in three epileptics between the ages of 2 and 60 years have seizures due to NCC.[5,6,7,8]

In most endemic countries NCC is mainly a multi-cyst disease (MNCC), whereas in India, solitary cysticercus granuloma (SCG) is the common presentation.[9]

Hospital data from different parts of the country indicate that more than 60% of NCC patients present with a single cyst lesion and one third with 2 or more cysts.[9,10,11] This is interesting because with similar risk factors in other endemic countries in South America and Africa, MNCC is more prevalent than SCG.

In South America the prevalence of *Taenia*sis is reported to reach 6% in the population, while porcine cysticercosis can be as high as 30%. The prevalence of t*aenia*sis in the general population in India is reported to be 0.1 – 6%[12] and 18.6% in certain pockets[13] whereas the prevalence of porcine cysticercosis is reported to be 10-26%.[14] These prevalence rates although less than South America,[15] are not that low given the magnitude of *T solium* egg production. Therefore in addition to prevalence levels of t*aenia*sis, other factors that may contribute to NCC disease profile in India like host immunity which is discussed later.

Pathogenesis – pathological stages

After entering the central nervous system, the live cysticerci induce little local inflammatory changes but usually grow in size. Cysticerci secrete a serine protease inhibitor called "*taenia*statin", which inhibits leukocyte migration, complement activation, and cytokine release. At this stage, called the **vesicular stage**, the cystic vesicles, round or slightly oval in shape, with translucid walls and diameters ranging from 0.5 to 1.5 cm, contain clear vesicular fluid. It also contains a larva or scolex, which can be seen as a small eccentric solid granule. The cysticercus is surrounded by a thin layer of fibrous tissue that separates it from the surrounding tissue. Electron microscopy shows microvilli in its surface. **Racemose cysticercus** variety looks like a large, lobulated vesicle, similar to a bunch of grapes. It may measure up to 10 cm and contain several milliliters of fluid. It is generally observed in ventricular cavities and in the cisterns located on the base of the cranium. Scolex can be demonstrated histologically only in such cysts.

Following cysticidal treatment or successful immune response, the first sign of involution of the cysticerci is the **colloidal stage**, in which a viscous and turbid fluid replaces the transparent fluid and the scolex dies. Colloidal cysticerci are surrounded by a thick collagen capsule, astrocytic gliosis, edema and neuronal degeneration. The scolex in the cyst is transformed into coarse mineralized granules – the **granular-nodular stage**. Finally the dead cyst is replaced by focal fibrosis and mineralization – the **calcific stage**.[16]

Meningeal cysticerci in particular elicit an intense inflammatory reaction in the subarachnoid space, forming exudates composed of lymphocytes, eosinophils, hyalinized parasite debris, and collagen fibers. This may result in obstruction of the CSF pathways causing hydrocephalus. Small penetrating arteries arising from the circle of Willis are frequently affected by the subarachnoid inflammatory reaction, which may cause occlusion of the vessel and cerebral infarctions. Ventricular cysticerci attached to the choroid plexus or ventricular wall also cause an intense inflammatory reaction, resulting in granular ependymitis and hydrocephalus. Immunocompromised states may cause heavy parasitism or activation of dormant cysts.

Neurocysticercosis: Classification

Neurocysticercosis is classified according to anatomic location as well as by developmental stage of the cyst.

The anatomical classification

i) **Parenchymal NCC:** Presents with seizures, space occupying effects and intracranial hypertension with breach of the blood brain barrier.[17,18]

ii) **Subarachnoid NCC:** Presents with meningitis, space occupying effects and hydrocephalus.[19]

iii) **Ventricular NCC:** Presents with acute hydrocephalus and meningitis.

iv) **Spinal NCC:** NCC can also locate in the spine.[20]

The influence of anatomic location on developmental outcome of cysts is also seen between the brain and muscle. Cysts in the immunologically privileged brain remain vesicular for longer periods than those in the muscle.[21]

Classification based on stage of the cyst

i) **Active NCC:** Viable cysts that do not produce symptoms and transitional forms when degenerating cysts produce acute symptoms

ii) **Inactive NCC:** Calcified cysts.[21,22]

Epidemiology

T solium infections are those of antiquity. Tapeworms and their treatment were discussed in ancient Egypt and possibly referred to in ancient Indian literature. The earliest description of cysticercosis are that of vesicles in the corpus callosum by Paranolus in 1550.[23] The modern biology of *T solium* began in Europe in the 1600's although it was only around 1850 that the link between the adult tapeworm and cysts were established, in ethically controversial studies, by Fredrich Kuchenmeister in Germany. He fed convicts awaiting execution with infected pork and in the autopsies performed after execution he detected growing and adult worms in the intestines of his subjects.[24]

The association of seizures and neurocysticercosis was documented in 1862 by the German psychiatrist Griesenger. In 1911 Vosgein described

cysticercosis in 807 people, most of them French soldiers who had lived in the colonies. Between 1930 and 1960 seminal studies on cysticercosis and seizures in British soldiers returning from India were reported by MacArthur, Dixon and Lipscomb.[25 - 27] This knowledge of cysticercosis has benefited from both the penal system and colonialism.

The indication that *T solium* infections are prevalent in large parts of the world emerged from epidemiological studies in the last twenty years, when radiological neuro-imaging and serological tests for cysticercosis became available.[28-30] *T solium* infections are the most common parasitic infestation of the CNS in endemic countries. They are considered re-emerging infections of public health magnitude and importance.

A brief overview of the epidemiology of human cysticercosis follows in which the prevalence of cysticercosis is taken to reflect levels of NCC that might exist in the regions studied.

Cysticercosis is endemic in some parts of all continents except for Australia and Antarctica. It is endemic to South, Central and Latin America, Sub-Saharan Africa and East and South Asia. In these areas, the disease accounts for up to 12% of all admissions to neurological hospital services and is the most common cause of acquired epilepsy in adults.[31] It is also seen in industrialized world as immigration from endemic countries rises.

Risk factors for transmission of cysticercosis in all countries include free roaming pigs, poor sanitation and a warm humid climate that permits egg

survival.[32-35] Human cysticercosis occurs where porcine cysticercosis is significant. In addition to these common risk factors there may be conditions that are specific to a country which contribute to the infection. These may include frequency of pork consumption in different populations, culinary habits (undercooking of meat, consumption of raw meat) and feeding patterns of free roaming pigs, as when pigs are fed human feces.

Country specific risk factors pertaining to India may include pig rearers who live together in specified areas of a village and form nodes for the transmission of infection.[36] In support of this are studies by Prasad et al[13] who found a high prevalence of *Taenia*sis among pig rearers (18.6%) in North India. Regions in endemic areas that are cysticercosis free are associated with a lack of pig industry, no pork consumption and / or good sanitation.[37]

The epidemiology of cysticercosis has been well studied in most countries of South and Central America and in Mexico. Seizures are associated with NCC in all these countries and it is the most common cause of recently acquired seizures. Young adults among all ages are the most infected. Increased infection levels seen with age are surmised to result from re-infections because of high levels of the parasite in the environment.[5,38]

As among the other countries of the Americas, Mexico has a high frequency of *T solium* infections. Approximately, 10% of the population in endemic areas is seropositive for cysticercus antibodies.[39] Epidemiological studies also indicate the importance of clinically silent NCC in Mexico.

Asymptomatic NCC, evidenced by calcified cysts on neuro-imaging, occurred in 9.1% of rural Mexico. Not all cases were seropositive nor did they correlate with exposure factors.[40] Prevalence rates of NCC therefore need to be viewed with caution as under reporting may occur.

T solium infections are equally prevalent in Central America. In rural Honduras NCC accounted for 37% of epilepsy cases while 6.4% of the population had CT findings suggestive of NCC.[41] Seroprevalence for cysticercosis in Guatemala was as high as 17% in the rural population.[42] In a study from Ecuador 2.25% of a rural population were found to be infected with *T solium* while at least 43% of this population was seropositive for cysticercus antibodies which indicates high exposure to the parasite.[43]

Although food habits differ across Brazil, the largest country in South America where raw or undercooked pork sausages are often eaten.[44] It is therefore not surprising that NCC is endemic in Brazil and accounts for up to 13% of neurological admissions to hospital. Over a quarter (27.8%) of all cysticercosis patients are elderly.[45] It is mandatory to test for NCC in epileptics in Brazil as 16% of them are considered to have NCC.[46] Seroprevalence of cysticercosis in the general population is estimated to range from 0.87 to 6.22% in the different states.[47]

Comprehensive epidemiology from Peru indicates the country is hyper endemic for cysticercosis and the cause for 20-30% of adult onset seizures.[48] Seroprevalence in the Peruvian Highlands, determined by immunoblots for

cysticercus antibodies, ranged from 7.1 to 26.9% in a background of 42 to 75% porcine cysticercosis. A similar prevalence of 24.2% prevailed in the coastal regions.[49] Clustering of seropositive individuals in households as well as around tapeworm carriers are important aspects of infection in Peru.[50] This phenomenon has been noted in Mexico as well.[38]

In Venezuela seroprevalence of cysticercus antibodies ranged from 4-36.5% in rural communities while active infections were apparent in 5.7-9.1% of these populations.[51] In countries of Africa where epidemiology of cysticercosis has been studied, the prevalence of *T solium* cysticercosis is high and a major cause of epilepsy. It is likely that cysticercosis will rise in Africa as the porcine population in most of Sub-Saharan Africa is large, on the increase and since pork is a significant component of the diet.[52]

In Western Cameroon (West Africa) where *Taenia*sis occurred in 0.13% of the population and cysticercosis in 11% of pigs, 3% of the populations were actively infected with *T solium* of whom 59.1% had NCC.[53] In other regions of Cameroon 1.2% of epilepsy cases were due to active infections of *T solium* although specific antibodies to the parasite were present in 44.6% of epileptics.[54] In Togo and Benin the prevalence of cysticercosis in the population was noted to be 2.4% and 1.3% respectively.[55]

Central Africa is hyper endemic for cysticercosis. *T solium* cysticercosis underlies the majority of epilepsy in Burundi. Active infection was found in 20% of

the seizure-free population but levels of infection are possibly higher as 32% of the population with seizures were positive for cysticercus antibodies.[56]

In Eastern and Southern Africa the prevalence of porcine cysticercosis is significant with more than 50% of pigs infected in some regions. High levels of porcine cysticercosis are associated with increased human NCC and although levels are not known, it is likely the prevalence of human cysticercosis is high in East and South Africa. This suggestion is supported by the estimated 34,662 NCC-associated cases of epilepsy in Eastern Cape Province in 2004.[57]

Epidemiological studies show that neurocysticercosis is endemic in all countries of Asia where risk factors for the infection prevail. In China seroprevalence of cysticercus antibodies ranged from 3.97-16.4%.[58,59] In South East Asia among the indigenous people of Papua in Indonesia 67% of epilepsy cases were due to NCC, a consequence of the import of the disease among these tribes.[60] In Bali 13.5% of epileptics were infected with *T solium* while 12.5% of the populations were asymptomatic for cysticercosis but seropositive for the antibodies.[61] Cysticercus antibodies were present in 2.2% of a rural population of Malaysia.[62] Active cysticercosis infection affect 5.35% of a mountainous population of Vietnam and 0.06% of those on the coast while urban populations of Vietnam do not seem to be infected.[63]

In India NCC occurs across the country and accounts for 8-31% of epilepsy cases among hospital patients. The prevalence of active epilepsy in the community was found to be 3.83/1000 people with one third of the epilepsies

suffering from NCC.[64] Cysticercus antibodies were estimated to be present in 17.3% of the population in North India with a higher prevalence (20%) in the rural population compared to urban populations (8%).[65] In South India cysticercus antibodies were noted in 6.1% of the population.[66]

A seroprevalence of nearly 16% for cysticercus antibodies in Vellore district, TamilNadu, India, where one-third of all households have one or more members seropositive for the antibodies, is high and categorizes the district as hyper endemic for exposure to *T solium*. The immunoblot used in this study under-reports infections with a single cyst (60% sensitive) suggesting that the prevalence in Vellore district may be higher than stated. This is important, as almost one-third of all epilepsy in Vellore district is due to NCC.[67] The high seroprevalence among adults of the district may reflect the known increased susceptibility to infection with age.[39]

Prasad et al., 2007,[13] reported a prevalence of *T solium Taenia*sis of 18.6% in the study conducted in a rural pig farming community of North India; factors associated with *Taenia*sis on multivariate analysis were age above 15 years, history of passage of *taenia* segments in stool, undercooked pork consumption and poor hand hygiene. 6.6% subjects with epilepsy were identified. The study showed alarmingly high rates of epilepsy and *T solium Taenia*sis in the study community.

Epidemiologic data suggest that 30−40% of *Taenia solium* seropositive people become spontaneously negative without acquiring cysticercosis and only

one-third of *Taenia solium* seropositive individuals harbor cysts within the brain. Although false-positive results may explain some of these cases, they could also be clinically silent cysticercosis cases or people who spontaneously resolved or even avoided infestation. Evidence supporting these possibilities in human and animal cysticercosis has been published.[45]

IMMUNE RESPONSE IN *TAENIA SOLIUM* INFECTION

When a pathogen enters an immunologically competent organism, innate (nonspecific) and adaptive (specific) immunological responses of the host result in the destruction of the pathogen and/or of the host. The protective or pathogenic effectiveness of the innate response greatly depends on the generation of a nonspecific inflammatory phenomenon locally in the surroundings of the pathogen. The adaptive immune response rests on a systemic, selective clonal proliferation of lymphoid cells and their differentiation to effector cells type Th1 or Th2 with the subsequent production of various cytokines and plasma cells with the production of specific antibodies.[68]

The great diversity of clinical forms associated with neurocysticercosis in humans suggests its origin involves a complex participation of the immune system. Epidemiological studies of *T solium* infections indicate that the cyst persists in humans for long periods of time, often for years, and a large percent of infected individuals develop antibodies to the parasite but do not contract disease. The immunity the parasite elicits in humans is clearly important in the pathogenesis of infection.

In mammalian hosts helminths typically induce a Th2 immune response of IL-4, IL-5, IL-10 and IL-13, IgE with expansion and mobilization of effector cells such as mast cells, eosinophils, and basophils. They also down modulate immune cells to hypo-responsive state. Alternatively activated macrophages are also considered to suppress inflammation and promote the Th2 response in helminth infections. Thus in addition to molecular mimicry and self masking to evade expulsion, helminth parasites induce an anti-inflammatory, hypo-responsive environment that permits their long survival in hosts. Immune modulation orchestrated by helminths is greatly dependent on the structural complexity of their antigens especially glycosylation.[69-73]

The immune response in cysticercosis can be divided into three phases. During the very early events after oncosphere ingestion, the immune reaction type and rate will determine resistance or susceptibility to metacestodes establishment. This is followed by a long lasting immune response to the presence of living parasites that actively evade destruction by the host immune system. Finally the parasite is destroyed by an inflammatory reaction, both in the natural evolution of the infections or following anti-parasitic treatment.[2]

Humoral immune response

Ingestion and absorption of *T solium* eggs (invasive embryos / oncospheres) leads, in a few days, either to their destruction, mediated through host complement and antibody, or their establishment in host tissue and

differentiation into cysticerci (metacestodes). It has been proved for several *taenia* species (ie., *T Taeniaeformis, T ovis, T hydatigena*) that antibodies and complement are the protective components against the oncospheres.[74] If the immune response is elicited more slowly than the development of the immune evasion mechanisms exerted by the parasite, the metacestodes gets established making antibodies and complements no longer effective in destroying it. Thus, a competition between the establishment of the protective immunity by the host and the development of the evasion mechanisms by the parasite seems to occur during the initial days of infection (fig.4).

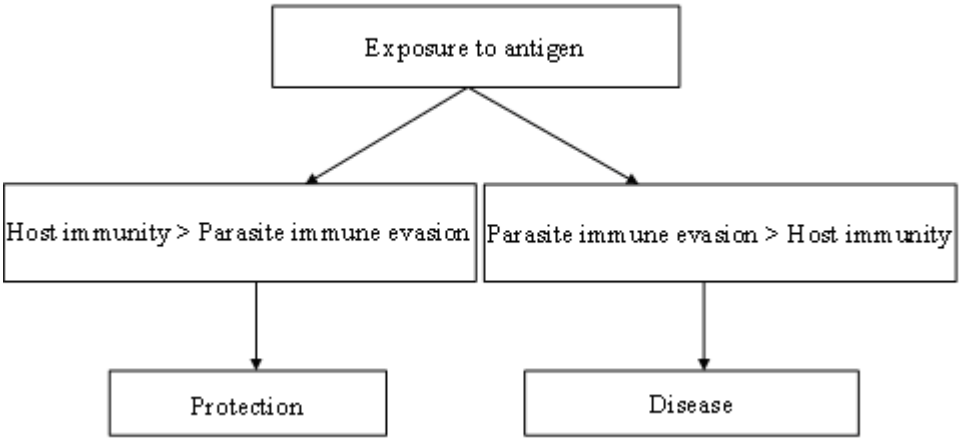


Fig.4

Studies have demonstrated that antibodies are protective during oncosphere invasion. The humoral response of *T solium* cysticercosis in the early phase of infection, driven by Th2 cytokines, is protective as it prevents infection by complement mediated antibody destruction of the invasive

oncosphere. It is shown in murine *T taeniaeformis* infections, that resistant strains produce specific antibodies earlier than susceptible strains.[75] These studies have led to the development of antigen vaccines that protect against infection in animal *T solium* cysticercosis.[76-78]

Antibody production also occurs during active infection although cysts themselves are resistant to antibody mediated destruction. Immunoglobulins coat the surface of the cyst and mask its antigens, which protects the parasite from the host.[79] The antigenic complexity to *T solium* leads to production of diverse immunoglobulins several of which are specific to the parasite. Most of the immunoglobulins are of the IgG class and reflect the chronicity of infection. The humoral response in *T solium* cysticercosis is both systemic and compartmentalized with intrathecal immunoglobulin synthesis occurring in NCC.[80-82]

Immune evasion mechanism

Once established, the viable metacestode despite transformation from a 30 micron long oncosphere to a 1cm diameter cyst, elicit minimal or no inflammatory reaction in host tissue due to their ability to evade host immunity. This also permits their survival in the host for long periods of time. Immune evasion strategies developed by *T solium* metacestodes include masking their surface with host derived antibodies and complement factors, molecular mimicry by synthesizing proteins that resemble host molecules and secretion of factors that actively suppress cellular and humoral immune responses.[83-86]

Nitric oxide

Resistance to *taenia* infection is also associated with induction of nitric oxide from macrophage that destroys the parasite. Mice susceptible to *taenia* infections were found to control parasitemia through induction of nitric oxide while inhibition of inducible nitric oxide synthase in macrophages increases parasitemia.[87,88]

Cellular immune response

Dying *T solium* cysts degenerate inducing infiltration and inflammatory reactions in surrounding tissue that lead to the granulomatous pathology of the infection. Reactions to degenerating cysts in the brain are associated with seizures. Predominant components of the inflammatory response include plasma cells, lymphocytes, eosinophils and macrophages. The degenerating parasite is phagocytosed by macrophages leaving a gliotic scar with calcification. Several correlative clinical, neuro-imaging, immunological and histopathological studies have demonstrated that symptomatic human cycticercosis corresponds to the presence of tissue inflammation around involuting cysticerci that are transiting between viable and calcified stages.[75,89,90]

In *T crassiceps*, Th1 immune response is shown to be protective during the early phase of infection and switch to Th2 type of response has been documented to occur 2-3 weeks later that down regulates the Th1 response.[91]

The cellular immune response of cysticercosis has been examined in the CSF, blood and granuloma itself.[92-94] In infected mice early granulomatous tissue expressed Th1 cytokines IL-2 and IFN-γ that caused parasite destruction. Subsequent to parasite destruction, down regulation of inflammatory reactions may occur through IL-4 that is detected in the tissue. The cellular immune response thus follows a temporal profile of Th1 in early pathology contributing to both pathogenesis of infection and clearing of the parasite that changes to a mixed Th1 / Th2 response when parasite destruction is complete.[95,96]

Viable cysts are associated with a Th2 response that is stimulated by the parasite. It is surmised that when cysts degenerate they no longer have the ability to direct the host to suppress Th1 immunity which is then activated and clears the parasite. In the brain the degenerating cyst is thought to activate microglia and resident macrophages to produce chemokines that attract monocytes and neutrophils across the blood brain barrier. This process initiates monocyte-astrocyte networks to further secrete chemokines that lead to influx of lymphocytes into the brain.[97] The lymphocyte influx and chemokine secretion directs Th1 cytokines to induce larval degeneration and mount antigen-antibody immune responses. This inflammatory Th1 granulomatous response is possibly that which gives rise to the symptoms of NCC. As the cyst is destroyed the response is modulated to express more Th2 cytokines to down regulate the inflammation.[98,99]

Immunosuppression by viable cysts is a prominent feature of cysticercosis. This immunosuppression is noted in brain tissue in the vicinity of

cysts, in the decrease of T and B cells in heavily infected pigs and anergy in mice of T cells in proximity to cysts.[91,100,101]

In lymphoproliferative assays active peripheral cellular immune responses are noted in patients with NCC not on immunosuppressive corticosteroids, which decrease in presence of high concentrations of specific *taenia* antigens. This would be in keeping with the immune suppression properties many *taenia* antigens possess.[102,103] Suppressed peripheral cellular immune reactions but increased levels of all specific IgG subclasses are noted in symptomatic NCC patients whose cysts are located in the ventricles or in the subarachnoid space. Cyst antigens from these locations drain into the CSF and serum and thus peripheral lymphocytes of these patients are exposed to high concentrations of *taenia* antigens.[104] Peripheral lymphocytes from asymptomatic NCC patients whose cysts are located in the parenchyma and are calcified are not exposed to high levels of *taenia* antigens and thus exhibit robust cellular immune reactions, predominantly of the Th2 type but low plasma levels of IgG. These studies were taken to indicate that a Th2 immune profile favors an uneventful course of infection and protects against the parasite.[105-107]

Protection was considered to develop in response to frequent contact with the parasite as may occur in endemic regions. In mice the Th2 cytokines IL-4 and IL-13 mediated host protection in *taenia* infections were found to require TNF-α for modulation. *Taenia* infections in TNF-α receptor knock-out mice were not eliminated although production of IL-4 and IL-13 were altered. Thus in endemic areas, vaccines that induce a Th2 response protect against the parasite.[68,108-111]

Clinical features

The cerebral manifestations of cysticercosis are diverse, related to the encystment and subsequent calcification of the larvae in the cerebral parenchyma, subarachnoid space and ventricles. Most often the neurologic disease presents with seizures, although many patients are entirely asymptomatic, the cysts being discovered radiologically. It is only when the cyst degenerates over many months or years after the initial infestation, that an inflammatory and granulomatous reaction is elicited and focal symptoms arise.[31]

Symptoms vary according to the location of the cyst, the parenchymal and extraparenchymal neurocysticercosis. The usual presentation of parenchymal neurocysticercosis is with seizures, which can be controlled with antiepileptic drug therapy. Occasionally, the cysts may grow and produce a mass effect. Extraparenchymal infection may cause hydrocephalus by mechanical obstruction of the ventricles or the basal cisterns, either by the cysts themselves or by an inflammatory reaction (ependymitis and/or arachnoiditis). The so-called racemose variety occurs in the ventricles or basal cisterns and is characterized by abnormal growth of cystic membranes with degeneration of the parasite's head (scolex).[112] These cases follow a progressive course, and even after ventricular shunting, the membranes or inflammatory cells and proteins frequently block the shunt.[113]

Diagnosis of Neurocysticercosis

The diagnosis of NCC is based on clinical findings, neuro-imaging (CT/MRI) and serological tests. Epidemiology can be helpful. The diagnosis can however be difficult as seizures, headache, focal deficits are common to several neurological disorders, neuro-images of cysticerci are not pathognomonic and serological tests are not always sensitive for infections with calcified or a low number of cysts.[114-124]

Neuro-imaging

The natural history of parenchymal cysticercosis has been studied by imaging studies using Computed Tomography (CT)[125,126] and Magnetic Resonance Imaging (MRI).[127](Fig.5,6) Viable cysts are 10 to 20 mm in diameter, thin-walled sacks filled with clear cyst fluid. On imaging studies, the wall is not visible and the fluid is isodense with the cerebrospinal fluid. There is little or no evidence of perilesional inflammation, and they do not enhance with contrast media on neuroimaging. As the parasite loses the ability to control the host immune response, an inflammatory process begins. Initially, the cysts show slight pericystic contrast enhancement. Later they become markedly inflamed and edematous and appear as ring-like or nodular areas of enhancement after the injection of contrast. This phase has been called “granulomatous cysticercosis, “cysticerci in encephalitic phase,” or “enhancing lesions.” Finally, the cyst is processed by the cellular response, and its remnants either are not detectable by imaging or become calcified lesions. “Giant” cysts, measuring more than 50 mm

in diameter, are occasionally found, located primarily in the sylvian fissure. Cysticercotic encephalitis is a rare form of the disease in which patients have numerous inflamed cysticerci, leading to severe diffuse cerebral edema. Extraparenchymal neurocysticercosis includes cysticerci in the ventricles and basal cisterns (racemose cysticercosis). Since the cyst membrane is thin and the fluid is isodense with the cerebrospinal fluid, uninflamed extraparenchymal cysticerci are usually not visible on CT and may only reveal subtle, indirect findings on MRI. Scans may reveal hydrocephalus without noticeable cysts, ependymitis, distorted basal cisterns, or basal meningitis.[113]

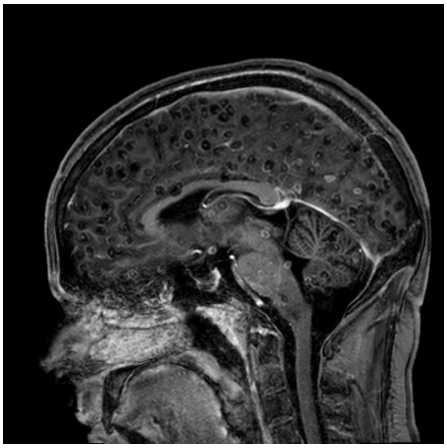


Fig. 5: Multiple NCC

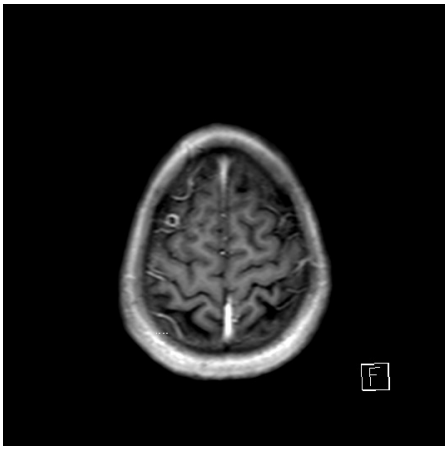


Fig.6: Solitary NCC

Serodiagnosis

Serological tests confirms diagnosis and are especially useful in differential diagnosis of NCC from tuberculomas, pyogenic brain abscess, mycotic granulomas, brain tumors and other conditions in which neuro-images can be similar. Serological tests are also valuable when radiological imaging is not available or too expensive. They have been important in epidemiology of cysticercosis. Cysticercosis demographics in most populations have largely been determined from serological data.[114,115,128-134]

There are numerous sero-immunological tests for *T solium* cysticercosis in several assay formats. There are assays to detect *T solium* antigens and cysticercus antibodies in saliva, blood, CSF and urine by ELISA and immune blots. Assays of blood, urine, and saliva determine both systemic and CNS cysticercosis. Tests for cysticercus antigens indicate an active infection while those for antibodies determine both past and current infections, as cysticercus antibodies persist for a long period of time (even for two years) after an infection.[135-141]

Serological tests for *T solium* cysticercosis are possible because of the immunogenic character of the parasite proteins. In *T solium* neurocysticercosis the immune response is determined by the number, stage of development and location of cysts in the brain and influenced by the immune suppressive properties of the antigens. Cysticercus antibody levels are stated to be decreased in infection of low cyst numbers and when cysts are calcified.[142-144]

Serological testing for cysticercosis began almost 100 years ago when in 1909 Weinberg demonstrated, by fixation, the presence of cysticercus antibodies in sera of pigs infected with “Cysticercus cellulosae”. The assay quickly moved into human testing. In 1911 Arthur Moses in Brazil using an aqueous extract of cysticercus cellulosae established for the first time cysticercus antibodies in the sera of 3 patients with cutaneous cysticercosis and in the CSF of one patient with encephalitic cysticercosis.[145,146]

Early serological tests mostly used crude antigen extracts for cysticercus antibody detection and consequently were associated with high rates of false positive and negative reactions.[147-149] Identification of specific *T solium* antigens, purification of antigens, use of recombinant DNA technology and hybridoma science have in the last two decades greatly improved these serological tests. Most assays in current use are sensitive and specific for *T solium* cysticercosis and their limitations of cross reactivity and false negative reactions well described.[150-160]

A large number of the immune assays use *T solium* glycoproteins as antigens given their immunogenic preponderance in the parasite. Antigenic glycoproteins useful for the detection of serum antibodies are mostly of small molecular weight (<50kDa). Cyst antigens of 13, 14, 18, 24, 38 and 50kDa were identified to be infection specific for *T solium* cysticercosis. Purification of these glycoproteins has been by classical methods of lentil lectin affinity chromatography and pH dependence.[161-163] CSF cysticercus serology has benefited from use of a very small protein of 10kDa.[164]

The complex glycosylation of *T solium* antigenic glycoproteins is essential for optimal serological performance. Recombinant *T solium* proteins have not performed as well as the native antigens in serological tests. The inability of recombinant systems to synthesize glycosyl units to the complexity required is stated to be the major reason for the lower performance of these systems. Work on high quality recombinant *T solium* antigens carries on unabated because of the immense advantage of using recombinant proteins over difficult to obtain native antigens, in serological tests required world wide for an infectious disease.[150,151,156,165]

Infectious disease is best diagnosed by the presence of the infecting organism. Monoclonal antibodies produced to excretory and secretory antigens of the parasite, which detect the antigens in circulation, provide this diagnostic criterion for *T solium* cysticercosis. It is natural that the seroprevalence of *T solium* cysticercosis, among patients and in populations, determined by the antigen assays are lower than levels obtained from determining prevalence of cysticercus antibodies.[51,117,166-170]

Serological assays for cysticercosis in current use are reliable for multi-cyst infections. In the natural, variable history of *T solium* cysticercosis, multi-cyst infections ensure sufficient antigens for immune stimulation. The tests are inadequate for infections with low numbers of cysts especially those categorized as solitary cyst infections of the CNS. In large parts of the world this does not matter as *T solium* infections are predominantly multi-cystic. In countries where *T*

solium infections of the CNS are common but of low cyst number, as in India, the assays must be improved.[5,7,8,10,114,115,124]

Diagnostic criteria

Diagnostic criteria for NCC currently suggested are given in Table 1, and classified as absolute, major, minor and epidemiological criteria. The criteria among these four groups required for a definite or probable diagnosis of NCC are given in Table 2. CT criteria for solitary cysticercus granuloma are well established and have a sensitivity and specificity of 99% (Table 3).[5,7,8,10,171]

Table 1: Diagnostic criteria for neurocysticercosis[171]

Categories of criteria	Criteria
Absolute	1. Histologic demonstration of the parasite from biopsy of a brain or spinal cord lesion 2. Cystic lesions showing the scloex on CT or MRI 3. Direct visualization of subretinal parasites by fundoscopic examination
Major	1. Lesions highly suggestive of neurocysticercosis on neuroimaging studies* 2. Positive serum EITB† for the detection of anticysticercal antibodies 3. Resolution of intracranial cystic lesions after therapy with albendazole or praziquantel 4. Spontaneous resolution of small single enhancing lesions‡
Minor	1. Lesions compatible with neurocysticercosis on neuroimaging studies§ 2. Clinical manifestations suggestive of neurocysticercosis# 3. Positive CSF ELISA for detection of anticysticercal antibodies or cysticercal antigens 4. Cysticercosis outside the CNS¶
Epidemiologic	1. Evidence of a household contact with *Taenia* solium infection 2. Individuals coming from or living in an area where cysticercosis is endemic 3. History of frequent travel to disease endemic areas

* CT or MRI showing cystic lesions without scolex, enhancing lesions or typical parenchymal brain calcifications

† Enzyme-linked immunoelectro transfer blot assay using purified extracts of *taenia solium* antigens, as developed by the Centers for Disease Control and Prevention (Atlanta, GA)

‡ Solitary ring-enhancing lesions measuring less than 20mm in diameter in patients presenting with seizures, a normal neurological examination and no evidence of an active systemic disease

§ CT or MRI showing hydrocephalus or abnormal enhancement of the leptomeninges, and myelograms showing multiple filling defects in the column of contrast medium

\# Seizures, focal neurologic signs, intracranial hypertension and dementia

¶ Histologically confirmed subcutaneous or muscular Cysticercosis, plain X-ray films showing “cigar-shaped” soft tissue calcifications or direct visualization of cysticerci in the anterior chamber of the eye

Table 2: Degrees of certainty for the diagnosis of neurocysticercosis[171]

Diagnostic certainty	Criteria
Definitive	1. Presence of one absolute criterion 2. Presence of two major plus one minor and one epidemiologic criterion
Probable	1. Presence of one major plus two minor criterion 2. Presence of one major plus one minor and one epidemiologic criterion 3. Presence of three minor plus one epidemiologic criterion
The presence of two different lesions highly suggestive of neurocysticercosis on neuroimaging studies should be considered as two major diagnostic criteria. Positive results in two separate types of antibody detection tests should be interpreted only on the basis of the test falling in the highest category of diagnostic criteria	

Table 3: Clinical and CT criteria for the diagnosis of an SCG[9]

All criteria must be satisfied to make a diagnosis of SCG

Clinical features	– patient should present with seizures – there should be no features of persistent raised intracranial pressure – there should be no evidence of a progressive neurological deficit, and – there should be no evidence of a systemic disease such as a primary malignancy, pulmonary or systemic tuberculosis and focus of pyogenic infection.
CT features	– the lesion should be solitary – the lesion should enhance with contrast injection – the lesion should measure less than 20mm in maximal dimension, and – edema may or may not be present around the lesion but if present should not be severe enough to cause a shift of the midline structures.
Proposed additional criteria	1) Epidemiological- history of residence in or travel to regions of the world endemic for cysticercosis; 2) Immunological- a positive enzyme linked immunotransfer blot assay for cysticercus antibodies in serum or CSF.

SCG – Solitary Cysticercal Granuloma CSF – Cerebro Spinal Fluid

MATERIALS AND METHODS

Study design

Peripheral blood mononuclear cells from neurocysticercosis patients and healthy controls were isolated and stimulated in culture for 24 hours with *T solium* infection specific glycoproteins of 13, 14, 18, 24, 38 and 50kDa, their N-glycan and peptide components separately. The culture supernatants were assayed for IL-12 as a marker for Th1 inflammatory response, IL-4 as a marker for Th2 anti-inflammatory response and nitric oxide. The profiles were compared between healthy controls and patients, further between solitary and multiple cysticercosis patients to determine the immune response stimulated by *T solium* infection.

Approval

The study was approved by Institutional Review Board and Human Ethics Committee of our institution.

Subjects

Fourteen patients with NCC, (8 MNCC; 6 SCG) and 6 healthy controls were studied.

All patients were on their first visit to the Neurology service of the hospital. Detailed history and neurological examination was performed on all patients.

Serological test for cysticercal antibodies and contrast enhanced CT/MRI examination was performed on all patients.

Inclusion criteria

Patients with history of recent onset seizures (< 6 months), focal or generalized, raised intracranial tension or both who fulfilled the diagnostic criteria for SCG or MNCC mentioned earlier were included in the study.

Exclusion criteria

Known cases of epilepsy and those who had received cysticidal drugs or steroids were excluded from the study.

Controls

Asymptomatic, healthy volunteers among laboratory staff, matched for age to patients were included as controls; all of them were negative on the EITB for cysticercus antibodies.

Experimental methods

Enzyme-linked Immunoelectro Transfer Blot (EITB):

Cysticercal antibodies were assayed in serum by the EITB method. A sample was considered positive for cysticercal antibodies on the appearance of immune bands to one or more of the seven *T solium* infection specific cyst glycoproteins of molecular weight 13kDa - 50kDa.[114]

Peripheral blood mononuclear cell cultures

Twelve ml venous blood was drawn from all subjects with informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated from blood. One million PBMCs in RPMI 1640 / 10% newborn calf serum were seeded in 24 well culture plates and cells in each well, stimulated with one infection specific *T solium* glycoprotein antigen, its N-glycan and peptide components. Cells were cultured for 24 hours at 37ºC in 5% CO2 / 95% air (humidified). Culture supernatants were assayed for IL-12, IL-4 and nitric oxide (NO).

Cells from each subject were therefore separately stimulated with 21 components of each of the seven infection specific antigens.

Cytokine and NO assays in PBMC culture supernatants

PBMC culture supernatants were assayed for IL-4 and IL-12 by ELISA as described by the manufacturer. Briefly, microtitre plates pre-coated with anti-IL-12 / IL-4 polyclonal antibodies were incubated with culture supernatants and IL-4 or IL-12 detected with biotin conjugated monoclonal anti-IL-12 / IL-4 antibody followed by streptavidin-peroxidase.

Nitrate, the stable end product of nitric oxide, was assayed in culture supernatants by the Greiss reaction after reduction with copper-cadmium to nitrite.[172]

Statistical analysis

Data was analyzed for differences between controls and patients by the nonparametric Mann Whitney U-Test in consideration of small sample size. Tests were considered significant at $p < 0.05$. Statistical analysis was carried out using SPSS 13.0 software.

RESULTS

Patients: Fourteen patients were included in the study, 6 SCG and 8 MNCC. Thirteen of the 14 presented with recent onset seizures (<6 months). Two patients had intracranial hypertension, one with seizures and one without seizures; both had MNCC, numerous cysts (>100) in the brain (Table 4).

On brain imaging all patients had ring enhancing lesions of less than 2cm in size; stages of the cysts varied from vesicular, colloid to granular-nodular and all of them were parenchymal cysts. Serology was positive in all MNCC patients and in only 2 of the SCG patients (Table 4).

CSF analysis (Table 5) was done in 12 of the 14 patients. Cytology showed predominant lymphocytes in all patients. 3 patients had pleocytosis (80, 40 and 20 cells respectively), all three had elevated protein (111, 92 and 63 mg% respectively).

Table 4: Patient characteristics

Patients	No	Mean Age (years)	Sex M:F	Seizure		↑ ICT*	Imaging			Serology #	
				GTCS	Focal		Single cyst	<5 cysts	>100cysts	Positive	Negative
SCG	6	29	4:2	6	-	-	6			2 (33%)	3 (50%)
MNCC	8	31	7:1	4	3	2 (25%)	-	5	3	8 (100%)	-

*↑ ICT in 1 patient with seizure and 1 without seizure; # Serology not done in 1 SCG patient

Table 5: CSF characteristics

NCC Type	No	Cells/c.mm		Protein (mg%)		Sugar (mg%)
		<5	>5	<60	>60	

SCG	4†	2	2	4	-	N
MNCC	8	5	3*	4	4	N

†CSF not done for 2 SCG patients, *all 3 with pleocytosis had ↑protein

PBMC response to *T solium* antigens: IL-12 (Table 6)

IL-12 was secreted by PBMCs from both controls and NCC patients in response to challenge by *T solium* antigens. N-glycans were significantly stronger stimulants of the response than their corresponding glycoproteins. No significant response was observed in cells challenged with peptides of the glycoproteins.

Controls showed a significantly higher IL-12 response compared to NCC patients in response to challenge by pure *T solium* infection specific antigens.

IL-12 response was significantly higher in patients with MNCC compared to those with SCG, when stimulated with N-glycans and no significant difference was observed with glycoprotein antigen.

Table 6: Interleukin-12 levels in culture supernatants of PBMCs stimulated with *T solium* antigens

T solium glycoprotein	PBMC culture supernatant – IL-12 pgm/ ml (Mean ± SEM)							
	Control (n=6)		NCC (n=14)		MNCC (n=8)		SCG (n=6)	
	Glycoprotein	Glycan	Glycoprotein	Glycan	Glycoprotein	Glycan	Glycoprotein	Glycan
LLGP	6.68 ± 3.15	8.81 ± 4.23	18.51±4.12	36.43±8.18#	19.2 ± 6.38	39.3± 15.0	17.5 ± 6.60	32.5 ± 5.93#
50kDa	17.8 ± 11.4	16.9 ± 5.82	1.40±0.63	3.33±0.88#	1.15 ± 0.73	4.60 ± 1.46#†	1.73 ± 1.32	1.64 ± 0.55#
38 kDa	13.9 ± 7.14	12.4 ± 2.89	0.30±0.15	1.36±0.46#	0.14 ± 0.14	1.37 ± 0.78#	0.50 ± 0.31	1.34 ± 0.56#
24 kDa	37.7 ± 21.5	60.7 ± 26.8	**5.95±1.83***	13.83±2.96#	**5.64 ± 2.89***	15.8 ± 4.69	6.35 ± 2.81	9.47 ± 1.50#
18 kDa	7.07 ± 1.95	14.1 ± 4.13	**0.54±0.22***	2.28±0.70#	**0.3 ± 0.22***	3.04 ± 1.25#	**0.86 ± 0.47***	1.25 ± 0.17#
14 kDa	15.3 ± 6.20	23.8 ± 13.6	**3.12±1.00***	10.24±1.89	**2.93 ± 1.53***	13.3 ± 2.60†	**3.37 ± 1.61***	6.12 ± 2.36
13 kDa	20.1 ± 6.15	32.4 ± 12.8	**3.27±1.02***	11.14±2.23#	**3.15 ± 1.69***	13.4 ± 2.97	**3.43 ± 1.38***	8.00 ± 3.85#

* p<0.05 Compared to controls (Glycoprotein as antigen); # p<0.05 Compared to controls (Glycan as antigen)

†p<0.05 MNCC compared to SCG (Glycan as antigen)

PBMC response to *T solium* antigens: IL-4 (Table 7)

IL-4 was secreted by PBMCs from NCC patients but not from controls in response to challenge by *T solium* antigens. N-glycans were significantly stronger stimulants of the response than their corresponding glycoproteins. No response was observed in cells challenged with peptides of the glycoproteins. The IL-4 response was greatest in NCC patients when cells were stimulated with Lentil Lectin Glyco Protein (LLGP).

The IL-4 response was significantly greater from SCG than MNCC patients against lower molecular weight glycoproteins and the N-glycans (18, 14 and 13kDa components).

Table 7: Interleukin-4 levels in culture supernatants of PBMCs stimulated with *T solium* antigens

T solium glycoprotein	PBMC culture supernatant – IL-4 pgm/ ml (Mean ± SEM)							
	Control (n=3)		NCC (n=14)		MNCC (n=8)		SCG (n=6)	
	Glycoprotein	Glycan	Glycoprotein	Glycan	Glycoprotein	Glycan	Glycoprotein	Glycan
LLGP	0.1 ± 0.0	0.03± 0.03	**80.8±9.30***	76.7±10.5‡	**75.4 ± 17.2***	85.1± 17.7‡	**88.0±5.31***	85.4±5.62‡
50kDa	0	0.13± 0.16	**3.45±1.09***	9.56±6.17	2.36 ± 0.97	2.71 ± 0.84	**4.9±2.44***	19.1±15.3
38 kDa	0	0	**4.44±1.36***	7.65±3.26‡	**3.02 ± 0.96***	10.6 ± 6.94	**6.31±3.18***	13.8±7.71‡
24 kDa	0	0.73 ± 0.89	**17.5±5.86***	35.5±16.7	**7.35 ± 2.24***	7.99± 2.30	**31.0±12.7***	73.0±37.8
18 kDa	0.27 ± 0.16	0.42 ± 0.31	**8.45±3.53***	11.5±3.92	3.31 ± 1.34	9.92 ± 7.02	**15.3±8.221*#**	22.5±7.56‡†
14 kDa	0.42 ± 0.01	1.02 ± 1.02	14.3±6.0	22.2±8.30	5.90± 2.55	15.3 ± 9.59	**25.7±13.9*#**	43.9±17.0‡†
13 kDa	1.48± 0.44	2.23 ± 0.03	17.1±6.41	26.9±10.3	5.71± 2.68	28.7± 18.0	**32.4±13.6*#**	55.2±20.7‡†

* $p<0.05$ Compared to controls (Glycoprotein as antigen); # $p<0.05$ MNCC compared to SCG (Glycoprotein as antigen)

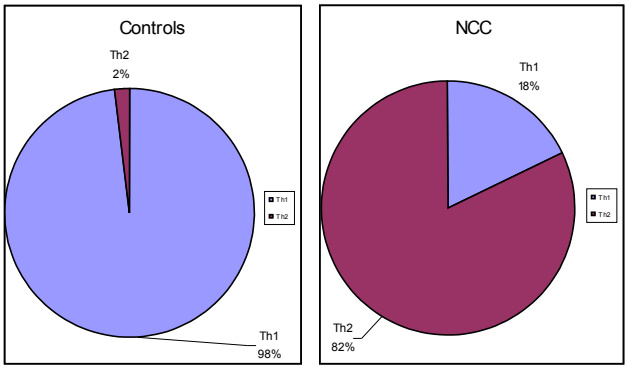
‡ $p<0.05$ Compared to controls (Glycan as antigen); † $p<0.05$ MNCC compared to SCG (Glycan as antigen)

PBMC Th1 / Th2 response in NCC

The relative Th1 and Th2 profiles of healthy controls and NCC patients to challenge by *T solium* infection specific antigens was determined from the total IL-12 and IL-4 response to the 50, 38, 24, 18, 14 and 13kDa antigens (i.e., to all infecting *T solium* glycoproteins)

The PBMC response to all infecting *T solium* glycoproteins shifted from pro-inflammation Th1 (98%) in healthy controls to anti-inflammation Th2 (82%) in all NCC, 88% in SCG and 67% in MNCC.(Fig.7) The response with glycans shifted from Th1 to Th2 from 97% in controls to 79% in all NCC, 89% in SCG and 67% in MNCC. (Fig.8)

Fig.7: Th1/Th2 response by PBMCs to *T solium* glycoproteins of 13-50 kDa



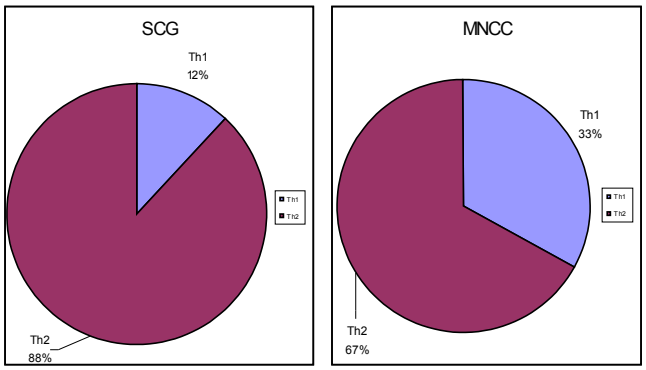
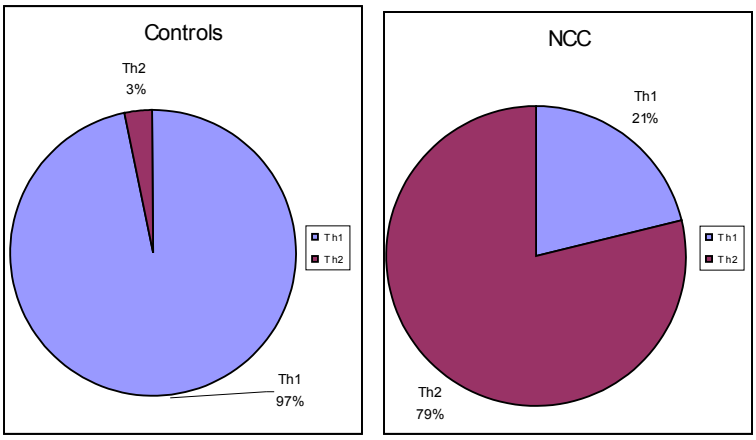
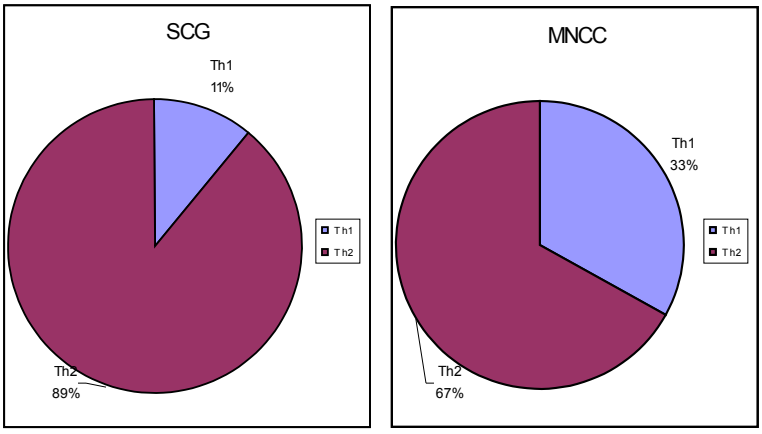


Fig.8: Th1/Th2 response by PBMCs to *T solium* glycans of 13-50 kDa





In controls the Th1 response was 51.6 fold higher than the Th2 response with glycoproteins and 35.4 fold higher with N-glycans.

In all NCC patients the Th2 response was 4.5 fold higher than the Th1 response in PBMCs challenged with *T solium glycoproteins*. This Th2 response

was 7.1 fold higher than the Th1 response in patients with SCG while only 2.1 fold higher in patients with MNCC.

In all NCC patients the Th2 response was 2.7 fold higher than the Th1 response in PBMCs challenged with *T solium* N-glycans. This Th2 response was 7.8 fold higher than the Th1 response in patients with SCG while only 1.5 fold higher in patients with MNCC.

Nitric oxide (Table 8)

NCC patients produced significantly higher nitrate than the controls, but there was no difference between MNCC and SCG patients. The N-glycans were stronger stimulants of nitric oxide production than the corresponding glycoproteins. The pure infection specific antigens were stronger stimulants of nitric oxide production than the LLGPs. No nitric oxide response was observed in cells challenged with the peptides of the glycoproteins.

Table 8: Nitrate levels in culture supernatants of peripheral blood mononuclear cells stimulated with *T solium* antigens. (glycoprotein component)

T solium glycoprotein	PBMC culture supernatant – nmoles nitrate / ml (Mean ± SEM)			
	Control (n=6)	NCC (n=14)	MNCC (n=8)	SCG (n=6)
LLGP	0.21 ± 0.05	**2.29 ± 0.73***	1.55 ± 0.53	**3.29 ± 1.64***
50kDa	14.35 ± 3.56	**92.84 ± 29.47***	**76.15 ± 19.98***	**115.1 ± 69.88***
38 kDa	9.80 ± 3.84	**47.17 ± 11.91***	41.73 ± 13.57	**54.43 ± 24.00***
24 kDa	4.99 ± 0.53	**98.18 ± 28.89***	**82.99 ± 20.38 ***	118.43 ± 68.23
18 kDa	7.18 ± 1.80	**22.11 ± 4.21***	24.53 ± 6.73	**18.9 ± 5.19***
14 kDa	11.83 ± 3.20	56.92 ± 14.99	64.40 ± 24.47	46.93 ± 17.44
13 kDa	13.45 ± 4.89	**95.54 ± 25.65***	**129.02 ± 42.08***	**50.89 ± 11.86***

* $p<0.05$ when compared to Controls

DISCUSSION

Development of neurocysticercosis following exposure may be influenced by the endemicity, number of exposures, egg-load and the presence of co-infections. The immune response of the host, both humoral and cellular also plays a major role in determining the outcome namely, clearance versus establishment of infection.

It has been shown in animals that immunity against helminth infections requires the induction of a Th1 (pro-inflammatory) response, while infection is established by induction of a Th2 (anti-inflammatory) response by the host.[91,173,174] *Taenia* infection in mice indicates a Th1 response protects the animals against the parasite, while a Th2 response is necessary to establish infection.[175] *T crassiceps* infected *BALB/c* mice developed an initial brief Th1 like response that is replaced by a strong Th2 response associated with parasitemia.[87] Nitric oxide and macrophage activation also contribute to resistance against *taenia* infections in mice.[88]

The cellular response has not been so clearly defined in human *taenia* infections especially neurocysticercosis, but may impact on disease profile. A predominantly Th1 pro-inflammatory environment that mostly clears infection may result in solitary cyst disease, while infections that are not cleared completely give rise to MNCC. This process would also be dependent on innate

immunity that is determined not only by the infecting organism but also modulated by co-infections and bystander antigens which differ between populations.

To explore the influence of host immunity and cyst number in NCC patients in Indian population, the response of PBMCs to infecting *T solium* glycoproteins was compared between healthy young adults and patients with multi-cyst and solitary cyst infections. The immune response to N-glycans of the infecting *T solium* glycoproteins was also studied in view of their critical importance for cysticercus antibody recognition. IL 12 was measured as a marker of the Th1 response and IL4 of Th2 response. The possible role of nitric oxide in protecting the host against infection and thus contributing to magnitude of cyst load was also studied.

Symptomatic NCC is associated with depressed cellular but active humoral responses. This has been noted in patients from Mexico.[104] In the present study all our patients presented soon after seizures and were considered to be symptomatic. They exhibited both active cellular and humoral immunity. In this study all controls with no cysticercosis (i.e., EITB negative) elicited a Th1 response and all patients, both SCG and MNCC, elicited a predominant Th2 response. This would support the idea of a pro-inflammatory cytokine milieu, produced in the early interaction of parasite with host that protects against parasite invasion and eliminates infection, while an anti-inflammatory environment allows for the survival of parasite and establishment of cyst formation. Our NCC patients, both SCG and MNCC had low levels of Th1

response (suppressed Th1 response) to establish infection. This is in contrast to an earlier study of cellular immunity in Indian patients with neurocysticercosis,[176] which showed a predominant Th1 response of PBMCs stimulated with crude soluble extract antigen and antigen B. The reason for this difference is not clear, but may be due to the nature of the different stimulating cyst antigens.

It is also possible that the relatively lower anti-inflammatory, but higher pro-inflammatory response noted with MNCC compared to SCG signify ongoing elimination reactions that the host does not perceive as necessary in a single cyst disease.

The results of the study suggest that non-infected host may be protected against the establishment of infection by robust pro-inflammatory reactions to infectious challenge. The establishment of infection is accompanied by suppression of pro-inflammation and supported by an anti-inflammatory response. Thus immunity in the present study group with NCC may transit from early protective, pro-inflammatory Th1 reactions to anti-inflammatory Th2 responses that permit parasite to survive in the host.

Cytokines associated with *T solium* cystic lesions in the brain are found to be largely pro-inflammatory Th1. This has also been seen in murine cysticercus granulomas.[90,97,174] It is not known if the compartmentalized CNS cytokine profiles in patients with NCC differ from those in periphery and whether CNS cytokines differ between patients with multiple versus solitary cysts and their impact on cyst load.

Helminth infections are considered to down regulate cellular immune response. This was not seen in the humoral response of the patients as most of them possessed antibodies to infection specific *T solium* glycoproteins. The Th2 response in infection, that permits parasite survival, may be accomplished by down regulation of the inflammatory response by immune suppressive cyst antigens. A similar understanding emerges from the CNS in murine cysticercosis where granulomas with dying cysts secrete Th1 cytokines that lead to clearing of parasite. As this occurs the response is modulated to Th2 expression, implicating down regulation of the granulomatous response.[174]

In animals protection against *taenia* infections is also provided by elevated NO, implying macrophages are necessary for protection against cysticercosis. Mice treated with nitric oxide synthase inhibitors are susceptible to *T crassiceps* infection and increased parasite loads.[88,177] In this study increased nitric oxide levels in NCC patients in response to *taenia* antigens may indicate NO toxicity to the parasite and implicate NO and macrophage in protection against cysticercosis.

In the present study even though we found predominant Th1 response in controls, with no significant Th2 response, there was no significant difference between the SCG and MNCC groups, both eliciting a dominant Th2 response. It is possible that the reacting cells resulting in cytokine secretion in the control group may be the infection naïve cells, thus representing the early phase in the infection, showing Th1 response at this particular point of time when the cytokines were examined. Had the cytokine examination been conducted over a

period of time with serial samples, it may have been possible to demonstrate the shift of the immune response from early protective Th1 to late permissive Th2 response. Similarly in the NCC group, both SCG and MNCC, the cells are probably mixed population with infection naïve and pre-exposed memory cells, leading to establishment of early shift from Th1 to Th2 response, (memory cells lead to more rapid and often more vigorous immune response) thus resulting in dominant Th2 response in both SCG as well as MNCC observed in our study.

In this complex immune response, a temporal profile of a wider repertoire of Th1 and Th2 cytokines of both naïve cells and memory cells in neurocysticercosis may help in elucidating the transition of Th1 to Th2 response in *taenia* infection.

CONCLUSIONS

1. Non-infected individuals mounted a strong pro-inflammatory response to *Taenia solium* infection specific cyst antigens.

2. Neurocysticercosis elicited predominant anti-inflammatory response to *Taenia solium* infection specific cyst antigens, which did not differ between solitary and multiple cyst infections.

3. Immunity in the present study group with NCC may transit from early protective, pro-inflammatory Th1 reactions to anti-inflammatory Th2 responses that permit parasite to survive in the host.

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APPENDIX

PROFORMA FOR COLLECTION OF DATA

Name of the patient: Age:

Sex: MALE/FEMALE Occupation:

Hospital No: Date:

Presenting complaints:

1) Seizures: Focal/GTCS/Secondary generalized
2) Raised ICT: Headache/ vomiting/ diplopia/ blurring of vision
3) Focal deficit: Cranial Nerves / Motor / Sensory / Cerebellar

Past history:

H/o seizures/epilepsy

Drug history:

H/o steroid/cysticidal drug intake

Examination findings:

General examination:

Subcutaneous nodules / Nodules in the tongue

Nervous system:

Higher mental function

Cranial nerves

Motor system

Sensory system

Cerebellum

Skull and spine

Investigations:

Complete Blood Count, ESR

Chest X ray

Serum creatinine, glucose

Serum cysticercal antibody (EITB)

Cerebrospinal fluid

-Cells:TC,DC,RBC

-Protein,glucose

-AFB smear/culture

-Routine smear/culture

-Fungal smear/culture

CT/MRI Brain with contrast

MASTER CHART

Pts	Sex	Age years	Clinical data			Past history	EITB	CSF			Imaging	
			Seizures		Raised ICT			cells	protein	glucose	No. of cysts	Stage of cysts
			Focal	GTCS								
1	M	41	**-**	Yes	**Raised ICT**	Nil	Positive	**80(L94)**	**111**	78	**Numerous**	V/G
2	M	21	-	Yes	Nil	Nil	Positive	3(L)	34	60	**4**	G/N
3	M	14	Lt focal	-	Nil	Nil	Positive	2(L)	20	46	**2**	C
4	M	64	Rt focal	-	Nil	Nil	Positive	5(L)	**63**	61	**2**	C
5	M	16	Rt focal	-	Nil	Nil	Positive	1(L)	28	63/77	**2**	C
6	M	38	-	Yes	Nil	Nil	Positive	**20(L98)**	**63**	57	**Numerous**	G/N
7	F	31	-	Yes	Nil	Nil	Positive	3(L)	23	72	**3**	G
8	M	26	**-**	Yes	**Papilledema**	Nil	Positive	**40(L98)**	**92**	57	**Numerous**	All
9	F	74	-	Yes	Nil	GTCS-1 yr back	**Not done**	1(L)	**56**	82/248	1	C/G
10	M	21	-	Yes	Nil	Nil	**NEGATIVE**	**Not done**			1	C/G
11	M	23	-	Yes	Nil	Nil	Positive	2(L)	27	74	1	C/G
12	F	18	-	Yes	Nil	Nil	Positive	**22 (L99)**	**47**	43/82	1	C/G
13	M	19	-	Yes	Nil	Delayed development	**NEGATIVE**	**15 (L99)**	37	78/130	1	C/G
14	M	18	-	Yes	Nil	Nil	**NEGATIVE**	**Not done**			1	C/G

ICT – Intra Cranial Tension GTCS – Generalized Tonic Clonic Seizures EITB – Enzyme linked Immuno Transfer Blot

CSF – Cerebro Spinal Fluid V – Vesicular C – Colloid G – Granular N – Nodular

Data for **IL12** secretion in PBMC - (IL12 pg/ml /ngm stimulant / million cells)								**MNCC (8)**		
GPS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	1	5.4	1.1	1.6	0	0	0	40	8.1	
	2	0	0	0	0	0	0	3.96	0	
	3	0	0.07	0	0	0.06	0	0.16	0.13	
	4	0	0	0	0	0	0	0	0	
	5	0	0	0	0	0	0	18.8	0	
	6	0.2	0	11.1	0	8	8.5	42	27.8	
	7	2.64	0	18.67	0.8	8.8	10.5	20.8	41.41	
	8	1	0	13.8	1.6	6.6	6.2	28	29.2	
	Mean	1.155	0.146	5.646	0.3	2.9325	3.15	19.215	106.64	153.72
	SD	1.945	0.386	7.650	0.595	4.074	4.496	16.886		
	SEM	0.735	0.145	2.891	0.224	1.539	1.699	6.382		
GLYCANS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	1	4	5.8	42	10.8	21.3	28	100	111.9	
	2	13.7	0.69	2.05	4.16	12.4	5.42	3.8	38.42	
	3	3.28	1.16	11.4	1.68	3.53	5.25	2.08	26.3	
	4	5.2	0.24	13.8	2.7	5.3	15.7	6.4	42.94	
	5	4.2	3.1	6.6	1	15.1	15.7	18	45.7	
	6	2.6	0	11.1	0.6	12.2	7.1	39	33.6	
	7	2.64	0	24.4	1.79	23.5	19.32	95.5	71.65	
	8	1.2	0	15.5	1.6	13.3	11.4	50	43	
	Mean	4.602	1.373	15.856	3.041	13.328	13.486	39.347	413.51	314.78
	SD	3.869	2.071	12.418	3.320	6.894	7.860	39.891		
	SEM	1.462	0.782	4.693	1.255	2.605	2.971	15.077		
PEPTIDES	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	1	0	0	0	0	0	0	0		
	2	0	0	0	0	0	0	0		
	3	0.016	0.006	0.009	0	0.004	0.004	0		
	4	0	0	0	0	0	0	0		
	5	0	0	0	0	0	0	0		
	6			NOT		DONE				
	7			NOT		DONE				
	8			NOT		DONE				
	Mean	0.003	0.001	0.001	0	0.0008	0.0008	0		
	SD	0.007	0.002	0.004	0	0.0017	0.0017	0		
	SEM	0.003	0.001	0.002	0	0.0008	0.0008	0		

Data for **IL12** secretion in PBMC (IL12 pg/ml /ngm stimulant / million cells)		**SCG (6)**		

GPS	Patients	50kDa	38kDa	24kDa	18kDa	14kDa	13kDa	LLGP	50-13kDa	LLGP
	9	0	1.45	5.4	0.64	2.04	1.94	8.4	11.47	
	10	0	1.34	2.55	0.24	0	1.88	1	6.01	
	11	7.6	0.25	2.5	1.5	2.53	2.57	9	16.95	
	12	0	0	0	0	0	0	17	0	
	13	1	0	11.1	0.1	8.2	8.5	40	28.9	
	14	1.8	0	16.6	2.7	7.5	5.7	30	34.3	
	Mean	1.733	0.506	6.358	0.863	3.378	3.431	17.566	97.63	105.4
	SD	2.965	0.695	6.294	1.053	3.621	3.097	14.765		
	SEM	1.326	0.311	2.815	0.470	1.619	1.385	6.603		
GLYCANS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	9	0	2.72	7.55	1.57	3.2	3.82	25.8	18.86	
	10	2.64	2.36	7.33	1.46	2.93	4	25.8	20.72	
	11	2.64	2.4	7.2	1.4	2.86	3.68	25.8	20.18	
	12	2.58	0.43	7.16	1.41	2.93	4.05	25.8	18.56	
	13	1.8	0.18	13.8	0.5	15.7	25.4	59	57.38	
	14	0.2	0	13.8	1.2	9.1	7.1	33	31.4	
	Mean	1.643	1.348	9.473	1.256	6.12	8.008	32.533	167.1	195.2
	SD	1.238	1.267	3.354	0.389	5.294	8.617	13.281		
	SEM	0.553	0.567	1.500	0.174	2.367	3.853	5.939		
PEPTIDES	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	9	0	0	0	0	0	0	0		
	10	0	0	0	0	0	0	0		
	11	0	0	0	0	0	0	0		
	12	0	0	0	0	0	0	0		
	13		NOT		DONE					
	14		NOT		DONE					
	Mean	0	0	0	0	0	0	0		
	SD	0	0	0	0	0	0	0		
	SEM	0	0	0	0	0	0	0		

Data for **IL12** secretion in PBMC (IL12 pg/ml /ngm stimulant / million cells)								**Control (6)**		
GPS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**

	15	11.6	9.45	27.7	7.9	15	16.5	0.18	88.15	
	16	64	43	131	13.7	42	46	1.9	339.7	
	17	31.2	18.5	44	10.2	15.5	21.1	0.038	140.5	
	18	0	0	2.08	4.44	6.66	20	15	33.18	
	19	0	0	8.44	2.04	3.77	6.85	8	21.1	
	20	0	12.45	13.33	4.16	8.88	10.71	15	49.53	
	Mean	17.8	13.9	37.758	7.073	15.301	20.193	6.686	672.16	40.118
	SD	25.701	15.976	48.090	4.361	13.869	13.772	7.062		
	SEM	11.494	7.145	21.506	1.950	6.202	6.159	3.158		
GLYCANS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	15	10.4	11.2	16.1	4	6.8	14.5	0.23	63	
	16	38.2	18.5	172	26	84	86	0.27	424.7	
	17	28	19	68	11.1	14.2	26.2	0.53	166.5	
	18	7	5.45	27.7	4.16	5.55	12.85	14	62.71	
	19	7	4.36	69.4	22.2	26.6	42.85	20	172.41	
	20	11.2	16.2	11.36	17.22	5.88	12.44	17.85	74.3	
	Mean	16.966	12.451	60.76	14.113	23.838	32.473	8.813	963.62	52.88
	SD	13.020	6.474	60.048	9.238	30.552	28.710	9.476		
	SEM	5.822	2.895	26.854	4.131	13.663	12.839	4.237		
PEPTIDES	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	15	3.68	3.68	5.6	6.24	3.3	4	0		
	16	3.36	2.7	2.7	1.6	3	2.7	0		
	17	1.22	2.6	2.5	1.7	1.5	1.1	0		
	18	0	0	0	0	0	0	0		
	19	0	0	0	0	0	0	0		
	20	0	0	0	0	0	0	0		
	Mean	1.376	1.496	1.8	1.59	1.3	1.3	0		
	SD	1.729	1.682	2.256	2.417	1.549	1.694	0		
	SEM	0.773	0.752	1.009	1.081	0.692	0.757	0		

Data for **IL4** secretion in PBMC - (IL4 pg/ml /ngm stimulant / million cells)								**MNCC (8)**		
GPS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	1	0	0	0	0	0	0	60	0	
	2	0.8	2.5	2.7	0.5	0.8	1.4	150	8.7	

	3	0	5.09	10.5	5.5	12.6	16.2	80	49.89	
	4	1.4	2.3	5	0.5	0.8	1.4	110	11.4	
	5	0.8	0.72	1.6	0.4	0	0	20	3.52	
	6	6.6	7.2	10.5	6.1	9.7	17.1	42.4	57.2	
	7	5.5	5.3	11.3	9.7	17.64	2.5	111	51.94	
	8	3.8	1.1	17.2	3.8	5.7	7.1	30	38.7	
	Mean	2.362	3.026	7.35	3.312	5.905	5.712	75.425	221.35	603.4
	SD	2.586	2.559	5.936	3.563	6.748	7.115	45.524		
	SEM	0.977	0.967	2.243	1.346	2.550	2.689	17.206		
GLYCANS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	1	0.6	2.5	3.8	1.6	3.3	4.5	75	16.3	
	2	1.4	2	3.8	1	3.7	20	160	31.9	
	3	0	56	2.72	55.5	77.7	145.7	75.6	337.62	
	4	4.6	4.7	15.5	3.3	6.2	8.5	105	42.8	
	5	3.4	3.4	2.7	1.1	2.4	3.4	25	16.4	
	6	6.8	5.6	5	5.5	8.8	14	49.2	94.9	
	7	2.9	5.3	15.4	8.4	11.32	20.5	141	204.82	
	8	2	5.6	15	3	9.3	13.7	50	98.6	
	Mean	2.712	10.637	7.99	9.925	15.34	28.787	85.1	843.34	680.8
	SD	2.230	18.382	6.097	18.584	25.398	47.669	46.989		
	SEM	0.843	6.947	2.304	7.024	9.599	18.017	17.760		
PEPTIDES	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	1	0	0	0	0	0	0	0		
	2	1.4	2	3.8	1	3.7	20	160		
	3	0	0.09	0.056	0	0	0	0		
	4	0	0	0	0	0	0	0		
	5	0	0	0	0.024	0	0	0		
	6		NOT		DONE					
	7		NOT		DONE					
	8		NOT		DONE					
	Mean	0.28	0.418	0.771	0.204	0.74	4	32		
	SD	0.626	0.885	1.693	0.444	1.654	8.944	71.554		
	SEM	0.313	0.442	0.846	0.222	0.827	4.472	35.777		

Data for **IL4** secretion in PBMC (IL4 pg/ml /ngm stimulant / million cells)								**SCG(6)**		
GPS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	9	10.4	7.6	55	52.7	88.8	92.8	80	307.3	
	10	0.6	0.54	7.7	8.8	16.6	22.8	92.4	57.04	
	11	3.4	20	61	9.4	17.7	21.4	80	132.9	
	12	13	4.36	55	8.8	15.5	30	92	126.66	
	13	2	1.8	6.6	5.5	4.2	21.4	108	41.5	
	14	0	3.6	1.1	6.6	11.5	6	76	28.8	
	Mean	4.9	6.316	31.066	15.3	25.716	32.4	88.066	694.2	528.4
	SD	5.459	7.125	28.580	18.383	31.293	30.610	11.893		
	SEM	2.441	3.186	12.781	8.221	13.994	13.689	5.319		
GLYCANS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	9	30	36	88	10.5	16.6	19.42	77	200.52	
	10	0	0	0	42.7	91.1	108	88.6	241.8	
	11	0	36	127.7	23.8	47.7	62.8	72.4	298	
	12	85	5.09	209	43.3	88.3	112.2	86.8	542.89	
	13	0	3.2	13.8	8	10.2	20.7	108	55.9	
	14	0	2.7	0	7.2	10	8.5	80	28.4	
	Mean	19.166	13.831	73.083	22.583	43.983	55.27	85.466	1367.51	512.8
	SD	34.411	17.248	84.706	16.915	38.062	46.359	12.582		
	SEM	15.389	7.7137	37.881	7.564	17.022	20.732	5.627		
PEPTIDES	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	9	0	0	0	0	0	0	0		
	10	0	0	0	0	0	0	0		
	11	0	0	0	0	0	0	0		
	12	0	0	0	0	0	0	0		
	13		NOT		DONE					
	14		NOT		DONE					
	Mean	0	0	0	0	0	0	0		
	SD	0	0	0	0	0	0	0		
	SEM	0	0	0	0	0	0	0		

Data for **IL4** secretion in PBMC (IL4 pg/ml /ngm stimulant / million cells)		**Control (3)**		

GPS	Patients	50kDa	38kDa	24kDa	18kDa	14kDa	13kDa	LLGP	50-13kDa	LLGP
	15	0	0	0	0.43	0.43	1.1	0.1	1.96	
	16	0	0	0	0.4	0.4	2.2	0	3	
	17	0	0	0	0	0.44	1.14	0	1.58	
	18		NOT		DONE					
	19		NOT		DONE					
	20		NOT		DONE					
	Mean	0	0	0	0.276	0.423	1.48	0.1	6.54	0.1
	SD	0	0	0	0.240	0.020	0.623	0.0		
	SEM	0	0	0	0.169	0.014	0.441	0.0		
GLYCANS	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	15	0	0	0	0	0	2.21	0.09	2.21	
	16	0	0	0	0.4	0.4	2.2	0	3	
	17	0.4	0	2.2	0.88	2.68	2.28	0.02	8.44	
	18		NOT		DONE					
	19		NOT		DONE					
	20		NOT		DONE					
	Mean	0.133	0	0.733	0.426	1.026	2.23	0.036	13.65	0.11
	SD	0.230	0	1.270	0.440	1.445	0.043	0.047		
	SEM	0.163	0	0.898	0.311	1.022	0.030	0.033		
PEPTIDES	**Patients**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**LLGP**	**50-13kDa**	**LLGP**
	15	0.016	0.032	0.016	0.032	0.048	0.064	0.03		
	16	0	0	0	0.016	0.016	0.064	0		
	17	0.016	0.06	0.04	0.032	0.032	0.048	0		
	18	0	0	0	0	0	0	0		
	19	0	0	0	0	0	0	0		
	20	0	0	0	0	0	0	0		
	Mean	0.010	0.030	0.018	0.026	0.032	0.058	0.01		
	SD	0.009	0.030	0.020	0.009	0.016	0.009	0.017		
	SEM	0.006	0.021	0.014	0.006	0.011	0.006	0.012		

MNCC(8) – Glycoprotein (GP) *NITRIC OXIDE*									
nmoles nitrite / ml / ngm protein / million cells (MNCC) (GLYCOPROTEIN)								**Total**	
Patients	**LLGP**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**50-13kDa**	**LLGP**
1	4.35	86	83	83	52	53	211	568	
2	1.8	80	56	188	37	80	114	555	
3	2.05	122	98.18	52.22	36.66	186.66	105.71	601.43	
4	2.05	166	43	77.77	28.88	0	108.5	424.15	
5	0	80	0	105	1.1	42.2	357	585.3	
6	1.6	56	36	111	26	128	108	465	
7	0.24	8	8.7	26	8.4	13	13	77.1	
8	0.35	11.2	9	21	6.2	12.4	15	74.8	
Mean	1.555	76.15	41.735	82.9987	24.53	64.4075	129.0262	**3350.78**	**12.44**
SD	1.4123	52.8756	35.9195	53.9321	17.8223	64.7544	111.3480		
SEM	0.5338	19.9851	13.5763	20.3844	6.73621	24.4748	42.0855		

MNCC(8) – Glycans (GL) *NITRIC OXIDE*									
nmoles nitrite / ml / ngm protein / million cells (MNCC) (GLYCANS)								**Total**	
Patients	**LLGP**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**50-13kDa**	**LLGP**
1	7.5	176	94.54	583	222	266	277	1618.54	
2	3.1	116	83.63	333	64	75.5	151	823.13	
3	2.85	166	74.54	316	103.33	193.33	260	1113.2	
4	2.85	100	40	55.55	103.33	134.28	145.71	578.87	
5	1.65	50	34.54	11	5.5	82	128	311.04	
6	5.64	24	17.4	40	15	71	34	201.4	
7	0.58	12.8	13.8	42.2	12	24	28.5	133.3	
8	0.39	6.4	5.4	40	7.5	13	17	89.3	
Mean	3.07	81.4	45.4812	177.5937	66.5825	107.3887	130.1512	**4868.78**	**24.56**
SD	2.4414	67.8876	34.3407	209.2521	75.3314	86.2845	101.0571		
SEM	0.9227	25.6591	12.9795	79.0898	28.4726	32.6124	38.1960		

MNCC(5) – Peptides (Pp) *NITRIC OXIDE*	
nmoles nitrite / ml / ngm protein / million cells (MNCC) (PEPTIDES)	**Total**

Patients	LLGP	50kDa	38kDa	24kDa	18kDa	14kDa	13kDa	50-13kDa	LLGP
1	0	0.8	0.96	1.6	3.6	0	3.2	10.16	
2	0	1.44	2.24	1.52	0	0.96	1.68	7.84	
3	0	1.28	1.6	0.4	0.4	2.32	2.32	8.32	
4	0	0.4	1.44	0.8	0	1.52	0	4.16	
5	0	0.16	0	0	0	0	0	0.16	
6			NOT		DONE				
7			NOT		DONE				
8			NOT		DONE				
Mean	0	0.816	1.248	0.864	0.8	0.96	1.44	**30.64**	**0**
SD	0	0.5496	0.8344	0.6960	1.5748	1.0007	1.4209		
SEM	0	0.2748	0.4172	0.3480	0.7874	0.5003	0.7104		

SCG(6) – Glycoprotein (GP) *NITRIC OXIDE*									
nmoles nitrite / ml / ngm protein / million cells (SCG) (GLYCOPROTEIN)								**Total**	
Patients	**LLGP**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**50-13kDa**	**LLGP**
9	2.2	52	96	155	32	97	65	497	
10	0.49	19.6	21	47	9.5	30	33	160.1	
11	3.9	156	145	411	34	60	88	894	
12	10.4	416	29	0	14	0	28	487	
13	1.85	24	21.8	66.6	17.7	82.22	68.57	280.89	
14	0.9	23	13.8	31	6.2	12.4	22.8	109.2	
Mean	3.29	115.1	54.4333	118.4333	18.9	46.9366	50.895	**2428.19**	**19.74**
SD	3.6807	156.2671	53.6856	152.5731	11.6165	38.9989	26.5400		
SEM	1.6460	69.8847	24.0089	68.2327	5.1950	17.4408	11.8690		

SCG(6) – Glycans (GL) *NITRIC OXIDE*									
nmoles nitrite / ml / ngm protein / million cells (SCG) (GLYCANS)								**Total**	
Patients	**LLGP**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**50-13kDa**	**LLGP**
9	3.8	52	100	255	53	131	105	696	
10	0.78	25.2	15	177	26	38.6	68.5	350.3	
11	4.1	144	158	455	110	200	205	1272	
12	4.2	34	83	211	86.66	166	231	811.66	
13	2.25	38	36.36	122	22.2	86.66	120	425.22	
14	1.16	19.2	18.18	57.7	11.5	22.2	66.2	194.98	
Mean	2.715	52.0666	68.4233	212.95	51.56	107.41	132.6166	**3750.16**	**16.29**
SD	1.5283	46.4285	55.9286	137.1328	39.4193	70.6941	69.8034		
SEM	0.6834	20.7634	25.0120	61.3276	17.6288	31.6153	31.2170		

SCG(4) – Peptides (Pp) *NITRIC OXIDE*									
nmoles nitrite / ml / ngm protein / million cells (SCG) (PEPTIDES)								**Total**	
Patients	**LLGP**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**50-13kDa**	**LLGP**
9	0	0.064	0.64	1.21	0.27	0.32	0.35	2.854	
10	0	0.54	0.43	0.48	0.76	0.56	0.36	3.13	
11	0	0	0	0	0	0	0	0	
12	0	0	0.8	0	0	0	0	0.8	
13			NOT		DONE				
14			NOT		DONE				
Mean	0	0.151	0.4675	0.4225	0.2575	0.22	0.1775	**6.784**	**0**
SD	0	0.2610	0.3465	0.5716	0.3583	0.2722	0.205		
SEM	0	0.1305	0.1732	0.2858	0.1791	0.1361	0.1025		

CONTROL(6) – Glycoprotein (GP) *NITRIC OXIDE*									
nmoles nitrite / ml / ngm protein / million cells (CONTROL) (GLYCOPROTEIN)								**Total**	
Patients	**LLGP**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**50-13kDa**	**LLGP**
15	0.108	7.652	1.568	5.876	4.572	3.788	4.048	27.504	
16	0.096	7.388	3.124	6.856	4.188	7.616	8	37.172	
17	0.105	8.888	1.956	3.91	3.464	4.8	8.888	31.906	
18	0.27	22.6	21.2	3.94	7	17.5	5	77.24	
19	0.36	25.6	16	5.2	13.7	19.1	26.8	106.4	
20	0.37	14	15	4.2	10.2	18.2	28	89.6	
Mean	0.2181	14.3546	9.808	4.997	7.1873	11.834	13.456	**369.822**	**1.405**
SD	0.1309	7.9738	8.5942	1.2003	4.0305	7.1753	10.9566		
SEM	0.0585	3.5660	3.8434	0.5367	1.8025	3.2089	4.8999		

CONTROL(6) – Glycans (GL) *NITRIC OXIDE*									
nmoles nitrite / ml / ngm protein / million cells (CONTROL) (GLYCANS)								**Total**	
Patients	**LLGP**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**50-13kDa**	**LLGP**
15	1.394	24.4	19.01	64.56	33.57	15.06	37.08	193.68	
16	1.5	18	38.18	147.22	36.66	51.1	68.56	359.72	
17	1.09	12	16.36	13.88	15.55	25.55	78.56	161.9	
18	0.22	33	18	61.66	15.66	31.33	22	181.65	
19	0.52	30.8	33.45	88	21.3	36.8	62.85	273.2	
20	0.24	36.4	24.7	75	11.7	34.2	42.28	220.52	
Mean	0.8273	25.7666	24.95	75.0533	22.4066	32.34	51.8883	**1389.68**	**4.964**
SD	0.5746	9.4067	8.9968	43.3758	10.3553	12.0084	21.5146		
SEM	0.2569	4.2068	4.0235	19.3982	4.6310	5.3703	9.6216		

CONTROL(6) – Peptides (Pp) *NITRIC OXIDE*									
nmoles nitrite / ml / ngm protein / million cells (CONTROL) (PEPTIDES)								**Total**	
Patients	**LLGP**	**50kDa**	**38kDa**	**24kDa**	**18kDa**	**14kDa**	**13kDa**	**50-13kDa**	**LLGP**
15	0.15	0.12	0.19	0.63	0.525	0	0.135	1.6	
16	0.14	0.272	0.4	0	0.592	0.56	0.672	2.496	
17	0.149	0.172	0.204	0.423	0.94	0.11	0.94	2.789	
18	0.27	0	0	0	0	0	0	0	
19	0.36	0	28	24	20	0	52	124	
20	0.37	0	0	0	8	0	0	8	
Mean	0.2398	0.094	4.799	4.1755	5.0095	0.1116	8.9578	**287.885**	**1.439**
SD	0.1082	0.1139	11.3671	9.7156	7.9364	0.2240	21.0897		
SEM	0.0484	0.0509	5.0835	4.3449	3.5493	0.1001	9.43164		

GPS – Glyco Proteins LLGP – Lentil Lectin Glyco Protein MNCC – Multiple Neuro Cysticercosis SCG – Solitary Cysticercal Granuloma

IL – Interleukins